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ACCUMULATION AND EFFECTS OF ORGANOTIN COMPOUNDS IN OYSTERS AND MUSSELS WITH SERUM BIOCHEMICAL AND CYTOLOGICAL FACTORS AND TISSUE BURDENS

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Oysters and mussels exposed to a concentration of 0–7 ppb (µg/liter) tributyltin from painted panels in flowing seawater accumulated tin in the digestive glands to comparable levels. The mussels experienced approximately 50% mortality during the 60–day test period, but the oysters suffered virtually no deaths. There was no evidence from either bivalve of elevated numbers of hemocytes during the test period and no evidence for cellular disruption as detected by increased levels of serum lysosomal hydrolases. Serum protein of exposed mussels relative to controls increased with time of exposure to the toxicant, while oyster serum protein, normally 10× higher than in mussels, did not. No evidence was found for elevated stress proteins (heat shock proteins) or metallothioneins in the serum hemocytes of either bivalve. Responses by these animals to fatal or near fatal doses of TBT were thus very different from responses to copper that we have reported elsewhere.[1,2]

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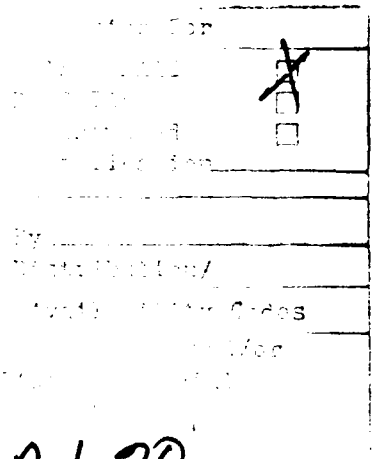
Accumulation and Effects of Organotin Compounds in Oysters and Mussels: Correlation with Serum Biochemical and Cytological Factors and Tissue Burdens



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Oysters and mussels exposed to a concentration of 0·7 ppb (μg/liter) tributyltin from painted panels in flowing seawater accumulated tin in the digestive glands to comparable levels. The mussels experienced approximately 50% mortality during the 60-day test period, but the oysters suffered virtually no deaths. There was no evidence from either bivalve of elevated numbers of hemocytes during the test period and no evidence for cellular disruption as detected by increased levels of serum lysosomal hydrolases. Serum protein of exposed mussels relative to controls increased with time of exposure to the toxicant, while oyster serum protein, normally 10 × higher than in mussels, did not. No evidence was found for elevated stress proteins (heat shock proteins) or metallothioneins in the serum hemocytes of either bivalve. Responses by these animals to fatal or near fatal doses of TBT were thus very different from responses to copper that we have reported elsewhere.[1,2]

We have been studying the cellular and biochemical responses of bivalves to organotin toxicants. In a 90-day test (60-day exposure, 30-day recovery) in flowing seawater in which panels painted with tributyltin-containing antifouling paints were immersed, we found serum chemical and cytological responses from bay mussels (*Mytilus edulis*) and eastern oysters (*Crassostrea*

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virginica) to be substantially different from responses to cupric ion that we have reported earlier[1] and at this symposium.[2]

In a concentration of 0·7 μg/liter TBT (expressed as the chloride) mussels sustained about 50% mortality, but oysters suffered no deaths although oyster condition indices were shown to decline (also consult Ref. 3 for a more detailed description of the experimental flow-through and dosing system).

Mussels were collected from San Diego Bay and oysters were purchased from a commercial source on Long Island, NY. Serum sampling procedures and analyses for hemolymph total protein and lysozyme were essentially as we have described previously.[1] DNA analyses as an analog of cell counts were performed according to the perchloric acid diphenylamine method of Cookson and Adams.[4] Hemocytes for metal analyses were pelleted from hemolymph in a clinical centrifuge (3400 rpm) for 45 s, the hemolymph was decanted and the pellets frozen awaiting analysis. Digestive glands were dissected from frozen test specimens, sonicated for 10 s, ground in a Potter–Elvehjem system in a minimal volume of 0·15% Triton X-100 in distilled water, and finally lyophilized in a spin freeze. The frozen hemocyte pellets and the lyophilized digestive glands were weighed, suspended in 1–1·5% HNO_3 containing 0·1–0·15% Triton X-100, and sonicated 10–15 s (pellet) or 30 s (glands). The hemocyte suspensions thus obtained were stable for several hours and were analyzed directly without further treatment. The sonicated digestive glands were spun for 60 s in a clinical centrifuge and the supernatants decanted directly into the atomic absorption sample cups. AAS for tin was carried out on a Perkin–Elmer Model 5000 using electrothermal atomization and graphite tubes fitted with L'vov platforms. Matrix modification of the samples in the graphite tubes was carried out essentially following the protocol of Pruszkowska *et al.*,[5] employing $NH_4H_2PO_4$ and $Mg(NO_3)_2$ in 1% HNO_3. Values for each sample were the average of triplicate determinations and final values were determined from regression lines obtained by standard additions run in actual samples and in the HNO_3-Triton X-100 sample diluent.

Results are summarized in Table 1. Oysters generally showed levels of serum protein an order of magnitude higher than mussels. Severely stressed mussels (as at this concentration) had serum protein significantly elevated above controls. Oysters at this dosage level showed a continually increasing serum protein level, but this was closely paralleled by the controls. The elevated mussel serum protein evidently was not associated with increased cell lysis since serum lysozyme did not increase. Both oyster and mussel hemocytes appeared resistant to being made 'leaky' by TBTCl as evidenced by the lack of significant increase in serum lysozyme in either animal. This is in contrast to results reported for cultured rat thymocytes and bone marrow

TABLE 1
Responses of Bivalves to Organotin Exposure[a]

	Hemolymph protein (μg/ml)		*Hemolymph lysozyme activity (mg/liter/s per ml)*		*Hemolymph DNA content[c] (μg DNA/ml)*	
	Start	*Depuration[b]*	*Start*	*Depuration*	*Start*	*Depuration*
Mussels (*Mytilus edulis*)						
Test	283 (116)	462 (307)	0·6 (0·1)	0·2 (0·02)	1·8 (0·8)	0·6 (0·4)
Control	170 (97)	44 (32)	0·4 (0·2)	0·1 (0·07)	3·5 (2·0)	0·2 (0·1)
Oysters (*Crassostrea virginica*)						
Test	1 738 (181)	3 727 (1 692)	0·8 (0·3)	0·6 (0·2)	1·5 (1·4)	0·7 (0·8)
Control	1 181 (826)	3 767 (1 391)	1·5 (1·2)	0·6 (0·07)	1·1 (0·6)	0·5 (0·3)

	Hemocyte tin uptake (ng Sn/μg DNA)			*Digestive gland tin uptake (ng Sn/mg dry wt)*	
	Start	*Highest*	*Depuration*	*Start*	*Depuration*
Mussels					
Test	3 (0·3)	48 (27)	21 (2)	0·5 (0·1)	3·9 (1·0)
Control	3 (1)	22 (4)	30 (3)	0·7 (0·3)	0·8 (0·2)
Oysters					
Test	10 (1)	48 (14)	37 (4)	0·3 (0·04)	2·8 (0·5)
Control	3 (1)	31 (11)	16 (3)	0·2 (0·2)	0·2 (0·6)

[a] Values are means of determinations made on 5 (categories 1–3) or 2–3 (categories 4 and 5) separate individuals. Numbers in parentheses represent sample standard deviations. Concentration of organotin (as TBTCl) was 0·7 μg/liter.
[b] Values were measured immediately prior to cessation of organotin exposure at the start of depuration.
[c] Hemolymph DNA content was determined on an aliquot of hemocytes and is an analog of total cell count. In our hands, 10^5 mussel hemocytes = 0·4 (±0·04) μg DNA and 10^5 oyster hemocytes = 0·6 (±0·16) μg DNA.

cells exposed to TBTCl.[6] Hemocyte numbers (based on DNA concentration measurements) showed no major differences between control and treatment animals in either bivalve, but total numbers declined in both control and test mussels throughout the test to one-fifth or less of starting levels while oyster counts showed a decline to one-half of starting values.

Digestive glands of treated mussels showed a gradual accumulation of tin while controls did not. Mussel hemocytes, however, showed no tendency for tin uptake for 30 days, then abruptly showed elevated levels that immediately began to decline while control hemocytes at that time began a

gradual tin accumulation, possibly from exposure to aerosoled toxicant. Mussel digestive gland tin burdens showed only a modest decline after 3 weeks of depuration. In contrast, oyster hemocytes in treated animals showed an early and rapid uptake of tin to levels significantly above controls. This elevation was maintained to depuration, then showed a second unexplained peak and a relatively rapid decline. Oyster digestive gland tin uptake was remarkably similar to that of the mussels (although slightly lower) both in apparent rate of uptake and in the magnitude of the burden achieved. Depuration from the oyster digestive gland during recovery also mirrored that shown by the mussels. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% gels) of hemocyte cytosol from both mussels and oysters showed no evidence for elevated metallothioneins or heat shock proteins induced by exposure to TBT.

Clearly these bivalves show biochemical and cytological responses to organometallics such as TBT that are very different from those displayed against metal ions such as Cu^{2+}. It is less clear that there are major differences between these two species in responses to TBT other than the differences in degree of response reported here. No single determination, or suite of determinations, has yet been identified, other than direct metal analyses, that are specific indicators of organotin intoxication.

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